

Aus der Medizinischen Universitätsklinik Basel  
(Vorsteher: Prof. Dr. med. H. Staub)  
Arbeit unter Leitung von PD. Dr. A. Pletscher

**Diagnostic Significance  
of 5-Hydroxyindole Acetic Acid Excretion**

Inaugural-Dissertation  
zur Erlangung der Doktorwürde  
der Medizinischen Fakultät der Universität Basel

vorgelegt von  
**EDWARD GRANOWITZ**  
von Greenwich, Conn. USA

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To my Mother and Father  
without their help and encouragement this work  
could not have been completed.



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5-hydroxyindole acetic acid (5HIAA), a normal component of the urine of mammal and man, is a major endproduct of 5-hydroxytryptamine (5HT) metabolism. The enzyme monoaminoxidase plays an important role in this degradation and is inhibited *in vivo* and *in vitro* by isopropyl-isonicotinic acid hydrazide (Iproniazid, Marsilid<sup>1</sup>) (1-9).

The physiological function of 5HT in the body still remains unclear. The amine has been claimed to be of importance in brain function, regulation of blood pressure, kidney function, and gastrointestinal motility, as well as in hemostasis. However, none of these functions attributed to 5HT has been proven.

Definite changes of 5HT metabolism were observed in patients with carcinoid who showed a greatly increased urinary excretion of 5HIAA (up to 500 mg or more per day). The 5HT content of the bloodplatelets was also elevated in such cases (10-17). These changes are probably due to a disturbance of the tryptophane metabolism, which results in a considerable 5HT production by the tumor. A much greater percentage of alimentary tryptophane seems to be converted in these cases to 5HT as compared to normal individuals (17).

With the exception of carcinoid, little is known about the excretion of 5HIAA in pathological conditions. Among approximately 60 cases with different physical diseases, excluding carcinoid, and four cases of schizophrenia, it was shown that six patients with collagen disease had lowered urinary excretion of 5HIAA. No definite changes of 5HIAA excretion were noted in the remaining patients as compared to normals (19). Also patients with renal hypertension and chronic glomerulonephritis showed subnormal levels of urinary 5HIAA (23). After oral loading with 2 grams of L-tryptophane in patients with larynx and bronchial carcinoma, the excretion of 5HIAA was elevated when compared with normal controls (18).

The purpose of this paper was to get more accurate information on 5HT metabolism by measuring urinary 5HIAA excretion in different physical diseases other than carcinoid, in mental patients, during pregnancy and labor, after surgical operation, as well as in the new-born. Furthermore, the effect of Iproniazid and dietary factors on the excretion of 5HIAA was examined.

### Method

1. The daily urinary 5HIAA excretion (mg/24 hours) in 100 patients with different physical diseases, 100 mental patients (35 with, 65 without Chlorpromazine medication), 3 normal pregnancies and 20 pregnancies with toxemia was determined.

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<sup>1</sup> trade name.

Also the urinary 5HIAA concentration ( $\gamma/\text{cc}$ ) of 11 women during labor, 12 new-born infants within the first 18 hours post partum, as well as 100 mental patients was assayed. 22 normal individuals of both sexes served as controls.

2. In two healthy women the 5HIAA concentration of the night urine was determined 7 times within a period of 8 weeks. Also the 5HIAA concentration in amniotic fluid of two cases was examined. Furthermore the total daily 5HIAA output in two patients before and two days following operation (1 cholecystectomy, 1 peptic ulcer) was assayed.

3. Three patients with chronic pulmonary tuberculosis each received daily doses of 8 mg/kg of Iproniazid over a period of 10 days. The total 5HIAA of 24 hour urines was determined every third day and compared to the 5HIAA excretion on 3 different days prior to Iproniazid medication.

4. Two individuals each received for two consecutive days, along with the regular hospital diet, 250 grams of lean beef. 5HIAA excretion prior to and during protein loading was compared. Three other persons each were administered 10 grams of L-tryptophane (primary substance for the biological synthesis of 5HT). The 5HIAA excretion was measured before and after ingestion of this amino acid.

5. To determine the interference of Chlorpromazine with the colorimetric method, the drug was added in various amounts to a standard solution of 5HIAA. 5HIAA determinations in these mixtures were made with the method mentioned below.

5HIAA was determined by the colorimetric method of *Udenfriend* and coworkers (1).

## Results

1. The daily total 5HIAA excretion in 22 normal individuals varied between 1.5 and 8 mg, the 5HIAA concentration in 24 hour urines between 0.8 and 7.3  $\gamma/\text{cc}$ . Mean value for the total daily output of 5HIAA:  $3.2 \pm 0.3$  mg/24 hours, mean value for the 5HIAA concentration:  $2.8 \pm 0.3$   $\gamma/\text{cc}^2$ .

2. In two normal controls, the daily 5HIAA concentration in the night urine varied between 1.5 and 5.1  $\gamma/\text{cc}$  (Fig. 1).

3. After supplement protein administration to the regular hospital diet, there was no change of 5HIAA excretion as compared to the control period. A small rise in 5HIAA output was noted after ingestion of 10 g of L-tryptophane in three patients. In fact the daily output of 5HIAA during the control period measured  $2.32 \pm 0.1$  mg, after tryptophane loading, it was  $4.0 \pm 0.1$  mg.

4. The 5HIAA excretion of patients with different physical diseases remained within the normal range (Fig. 2).

5. Of the 100 mental patients examined, 20 showed subnormal daily 5HIAA excretion. Also the incidence of low 5HIAA concentration was more frequent in the group of psychotics than in the normals (Fig. 3). 24% of the patients had urine concentrations of 5HIAA below the lower limit of normal controls (0.8  $\gamma/\text{cc}$ ). In one single case a somewhat increased 5HIAA output was observed (8.9 mg). No accumulation of subnormal daily 5HIAA values in a definite group of the examined mental patients could be seen. Also there was no appreciable difference between those patients receiving Chlor-

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<sup>2</sup> Standard deviation.

$\mu\text{g}/\text{cc}$  5HIAA

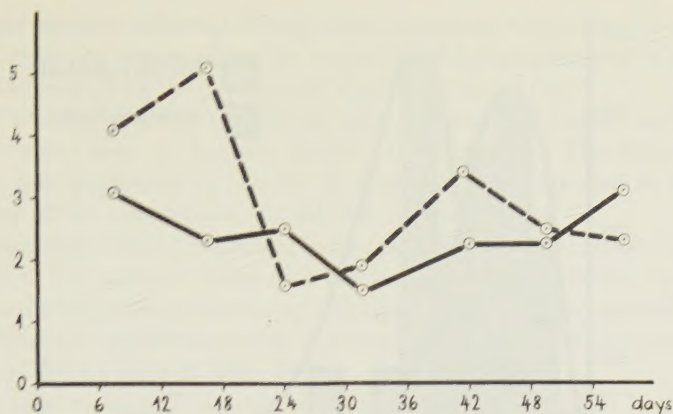


Fig. 1. Variation of 5HIAA excretion in two healthy women during a period of 8 weeks. Each dot represents the 5HIAA concentration of urine excreted during the night.

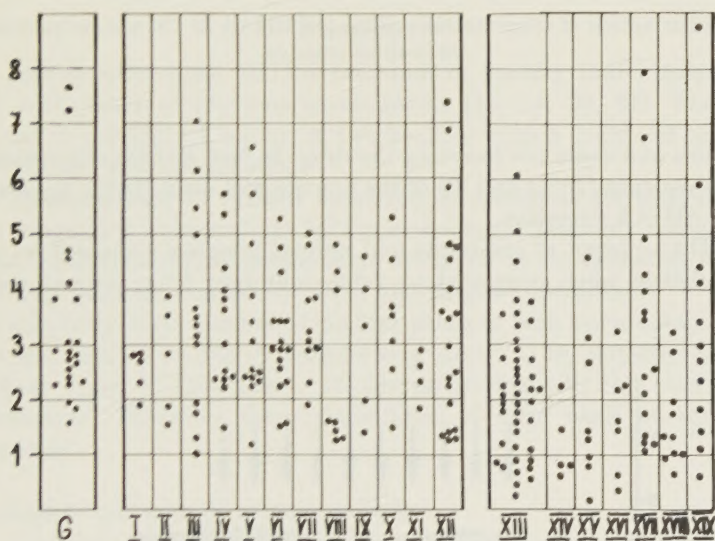


Fig. 2. Excretion of 5HIAA in 24 hour urine of healthy individuals and patients with different diseases. Ordinate: 5HIAA in mg/24 hours.

- |                                   |                                |
|-----------------------------------|--------------------------------|
| G Healthy individuals             | X Blood Diseases               |
| I Hypertension                    | XI Pulmonary Tuberculosis      |
| II Diabetes mellitus              | XII Pathological Pregnancies   |
| III Cardiac Disease               | XIII Schizophrenia             |
| IV Cancer                         | XIV Manic Depressive Psychosis |
| V Gastrointestinal Tract Diseases | XV Psychopathy and Neurosis    |
| VI Infectious Diseases            | XVI Oligophrenia               |
| VII Liver Diseases                | XVII Alcoholism                |
| VIII Kidney Diseases              | XVIII Arteriosclerosis cerebri |
| IX Allergic Diseases              | XIX Other Psychoses            |

Each point represents a 5HIAA value of a patient.

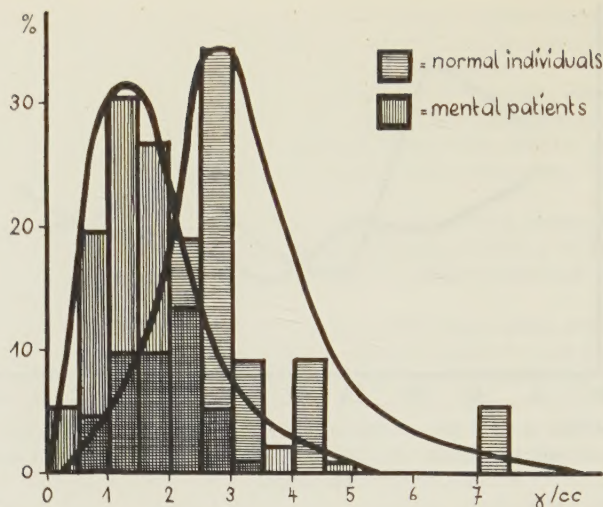


Fig. 3. Distribution of concentration values for 5HIAA in 100 mental patients and 22 healthy controls.

promazine and those not receiving the drug. In fact, 8 of the chlorpromazine treated patients (23%) and 12 of the non-treated ones (18%) showed sub-normal 5HIAA excretion.

6. 5HIAA output in toxicemia and normal pregnancy showed no appreciable change when compared to values obtained from normal controls.

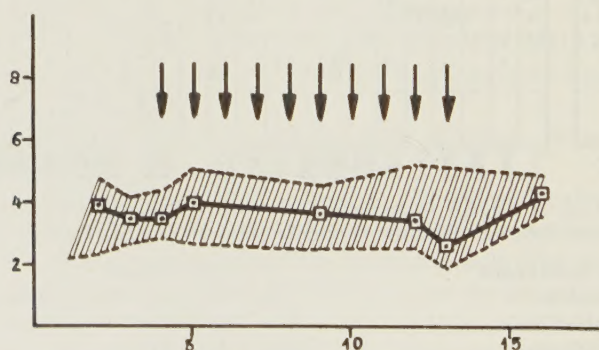


Fig. 4. Effect of isopropyl-isonicotinic acid hydrazide (Iproniazid) on 5HIAA excretion in three individuals. Ordinate: 5HIAA in mg/24 hours. Abscissa: Time in days. Arrow: Application to each of 8 mg/kg per os. Solid Line: Mean value for the three individuals. Dotted lines: Maximal and minimal values of the three individuals.

7. Urine portions collected during labor contained  $3.4 \pm 0.55$   $\gamma/\text{cc}$  5HIAA. The difference as compared to the mean 5HIAA concentration in urine of normal controls ( $2.8 \pm 0.3$   $\gamma/\text{cc}$ ) is not significant ( $p > 0.05$ ).

8. The average 5HIAA content in urine of new-born babies was higher ( $6.9 \pm 2.5$   $\gamma/\text{cc}$ ) than in healthy adults ( $2.8 \pm 0.3$   $\gamma/\text{cc}$ ). The difference is, however, not significant ( $p > 0.05$ ). In amniotic fluid collected at the time of rupture of the membranes, no 5HIAA was detectable.

9. The average 5HIAA excretion in two patients before and after operation was within the normal range (5.7 mg on the last day before operation, 2.7 mg on the first postoperative day, 3.9 mg on the second postoperative day).

10. Iproniazid application over a course of 10 days did not essentially affect the 5HIAA output (Fig. 4).

11. Chlorpromazine in a concentration range between 300 to 600 mg/1000 cc diminished the extinction of the reaction product of a standard 5HIAA solution between 4 and 8 percent.

### Discussion

The results concerning 5HIAA excretion of healthy individuals are in accord with those of previous investigators (13, 14, 20, 21). The daily variations of the 5HIAA output in two healthy women could not be correlated with the menstrual cycle.

The measurements of 5HIAA excretion in numerous physical diseases and in pregnancy indicate that 5HT metabolism is probably not affected in these conditions. Furthermore there seems to be no alteration of 5HT metabolism in stress, caused by labor, and surgical operation.

The relatively high number of mental patients with subnormal 5HIAA output can possibly be due to the following errors: a) Incomplete collection of 24 hour urine, b) Interference of chlorpromazine with the colorimetric determination. Therefore these possibilities of error shall be examined more closely.

#### *a) Incomplete collection of urinary samples:*

This possibility can be probably ruled out by the fact that not only the total output was low in 20% of the cases, but also the 5HIAA concentration ( $\gamma/\text{cc}$ ). The latter would probably not be affected to a relevant extent even if the 24 hour collection was incomplete.

#### *b) Interference of chlorpromazine with the method:*

Experiments with addition of various concentrations of chlorpromazine to a standard solution of 5HIAA showed that for chlorpromazine concentrations up to 600 mg/1000 cc, the interference with the method was probably negligible. Therefore in the cases there was probably no major error due to chlorpromazine. The therapeutic doses of the drug varied between 200 and 400 mg/day. Even if all the chlor-

promazine was excreted unchanged, the urinary concentration of the drug would probably not have exceeded 400 mg/1000 cc in the urine.

There is further evidence to substantiate that chlorpromazine did not disturb the method. In fact, the percentage of subnormal 5HIAA excretion practically was the same in the chlorpromazine treated as in the untreated group.

The reason for the relatively high incidence of subnormal 5HIAA output in mental patients is not clear and requires further investigation. Above all, it can not be decided whether this low output is due to a disturbance of 5HT metabolism in brain. The 5HT content of the brain represents only a few percent of the total 5HT content in the body. Little is known about the metabolic rate of 5HT in brain (22). Therefore it is well possible that subnormal output of 5HIAA does not reflect metabolic disturbances of the 5HT in the brain, but rather a disturbance in other organs with bigger 5HT content (gastrointestinal tract).

Bacterial activity in the gastrointestinal tract does not seem to be involved in the formation of 5HT and 5HIAA. This can be derived from the findings that the output of 5HIAA in new-born babies, who do not have bacteria in the intestinal tract, is not significantly different when compared with adult controls. Furthermore, nutritional factors do not affect 5HIAA output considerably. This is shown by the experiments with protein and tryptophane loading whereby the 5HIAA is altered but slightly. This is probably not an important source of error for diagnostic purposes.

The lack of influence of Iproniazid on 5HIAA excretion can possibly be explained by the fact that the relatively low therapeutic doses of the drug do not affect the 5HT metabolism of the large body depots. This explanation is substantiated by experimental results obtained from guinea pigs and rats, where the 5HT metabolism in brain after Iproniazid was definitely altered but in the intestine only slightly or not at all (24).

From the findings in patients with carcinoid as well as from the described results, it can be concluded that greatly increased urinary 5HIAA excretion in adults is most probably pathognomonic for carcinoid.

### Summary

The 5 hydroxyindole acetic acid (5HIAA) excretion in 100 patients with various organic diseases other than carcinoid, 3 normal and 20 pathological pregnancies, 11 women during labor, as well as 12 new-born babies, was not different from that in healthy adults.

20 out of 100 patients with various mental diseases showed subnormal 5HIAA values in 24 hour urines. Chlorpromazine medication did not appreciably interfere with the colorimetric method for the determination of 5HIAA. Surgical operations had no effect on the 5HIAA output. Amniotic fluid did not contain 5HIAA.

5HIAA excretion was not altered during Iproniazid treatment and slight changes produced by dietary factors were probably of no practical importance.

Marked increase of 5HIAA excretion is considered to be pathognomonic for carcinoid.

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